

**WHAT IS CLAIMED IS:**

1. A method of isolating a recombinant adeno-associated virus, comprising applying  
a sample containing recombinant adeno-associated virus to an iodixanol gradient,  
5 and collecting said recombinant adeno-associated virus from said gradient.
2. The method of claim 1, wherein said iodixanol gradient is a discontinuous  
gradient.  
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3. The method of claim 2, wherein said iodixanol gradient comprises an about 15%  
iodixanol step, an about 25% iodixanol step, an about 40% iodixanol step, and an  
about 60% iodixanol step.  
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4. The method of claim 3, wherein said recombinant adeno-associated virus is  
collected from said 40% iodixanol step.  
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5. The method of claim 3, wherein said 15% iodixanol step further comprises about  
1 M NaCl.
- 25 6. The method of claim 1, wherein said iodixanol gradient is subjected to  
centrifugation after applying said sample.
7. The method of claim 1, further comprising contacting said recombinant  
30 adeno-associated virus with a matrix comprising heparin, under conditions

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effective to permit binding of said virus to said matrix, removing non-bound species from said matrix, and eluting said virus from said matrix.

- 5      8.      The method of claim 7, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.
- 10      9.      The method of claim 7, wherein said matrix is comprised within an HPLC column.
- 15      10.     The method of claim 7, wherein said virus is eluted from said matrix with a solution comprising about 1 M NaCl.
- 20      11.     The method of claim 1, further comprising contacting said recombinant adeno-associated virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix, and collecting the non-interacting virus from said hydrophobic matrix.
- 25      12.     The method of claim 11, wherein said hydrophobic matrix comprises phenyl groups.
13.     The method of claim 12, wherein said hydrophobic matrix is phenyl-sepharose.

14. The method of claim 1, further comprising applying said recombinant adeno-associated virus to a cesium chloride equilibrium density gradient, and collecting said recombinant adeno-associated virus from said gradient.
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15. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with at least a first ion exchange chromatography medium, under conditions effective to permit interaction of said virus with said medium, removing non-interacting species from said medium, and eluting said virus from
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16. The method of claim 1, wherein said sample further comprises a virus.
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17. The method of claim 16, wherein said sample further comprises an adenovirus.
18. The method of claim 1, wherein said sample further comprises at least a first
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- polypeptide or protein.
19. The method of claim 1, wherein said sample further comprises a cell extract or a
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- growth medium.
20. A method of isolating a recombinant adeno-associated virus, comprising the steps of:

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- a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;
  - b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;
  - c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;
  - d) removing non-bound species from said matrix; and
  - e) eluting said virus from said matrix.
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21. A method of isolating a recombinant adeno-associated virus, comprising the steps of:
- a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;
  - b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;
  - c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;
  - d) removing non-bound species from said matrix;
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- e) eluting said virus from said matrix;
- f) contacting the eluted virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix; and
- g) collecting the non-interacting virus from said hydrophobic matrix.
22. A method for reducing or eliminating adenovirus from a recombinant adeno-associated virus composition contaminated with adenovirus, comprising applying a sample containing recombinant adeno-associated virus and adenovirus to an iodixanol gradient, and collecting from said gradient at least a first fraction comprising said recombinant adeno-associated virus.
23. A method of producing a recombinant adeno-associated virus having a particle-to-infectivity ratio of less than about 100 to 1, comprising the steps of:
- a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;
- b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;
- c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;
- d) removing non-bound species from said matrix; and

e) eluting said virus from said matrix.

- 5      24. Recombinant adeno-associated virus, prepared by applying a sample containing recombina nt adeno-associated virus to an iodixanol gradient, and collecting said recombina nt adeno-associated virus from said gradient.
- 10     25. A kit comprising, in a suitable container, iodixanol, a matrix comprising heparin and instructions for isolating recombina nt adeno-associated virus.
- 15     26. The kit of claim 25, wherein said iodixanol is formulated as an iodixanol gradient.
27. The kit of claim 25, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.
- 20     28. The kit of claim 25, further comprising a hydrophobic matrix.
- 25     29. The kit of claim 28, wherein said hydrophobic matrix comprises phenyl groups.
30. The kit of claim 29, wherein said hydrophobic matrix is phenyl-sepharose.